

Dual-Column Reconciliation IUPAC #		Placental Median (ng/g- wet)	% above LOD	Dual-Column Reconciliation IUPAC #		Placental Median (ng/g- wet)	% above LOD	
24+27	1	0.000	16.0%	105+141 123+149	89+101	0.066	87.8%	
	3	0.000	21.2%		97	0.020	84.6%	
	4+10	0.000	14.7%		99	0.063	99.4%	
	5+8	0.007	69.2%		132	0.007	61.5%	
	6	0.000	24.4%		109+147	0.024	78.8%	
	7+9	0.000	20.5%		114+133	0.009	94.2%	
	12+13	0.001	57.1%		118	0.089	98.1%	
	15+17	0.004	71.8%		131	0.003	82.1%	
	16+32	0.006	87.2%		128+185	167	0.010	90.4%
	18	0.003	75.6%		129+178	0.002	72.4%	
	19	0.000	36.5%		130+176	0.006	76.3%	
	20+33+53	0.005	59.6%		134	0.004	92.3%	
	22	0.001	58.3%		137	0.007	94.9%	
	25	0.000	39.7%		138+163+164	0.118	98.7%	
	29+54	26	0.000		24.4%	146	0.012	98.1%
28		0.016	96.8%	153	0.125	99.4%		
34		0.000	55.1%	157+173+201	156+171+202	0.038	98.7%	
31		0.001	54.5%	158	0.003	75.0%		
35		0.000	44.9%	170+190	0.026	99.4%		
41+64+71	37+42+59	0.012	82.1%	172	0.002	87.8%		
	40	0.002	73.1%	174	0.002	74.4%		
	43+49	0.006	86.5%	177	0.008	98.7%		
	44	0.022	84.6%	179	0.001	70.5%		
	45	0.000	50.6%	180	0.058	100.0%		
56+60+92	46	0.000	30.1%	183	0.008	97.4%		
	47+48+75	0.000	9.6%	187	0.012	99.4%		
	51	0.000	35.9%	189	0.000	53.2%		
	52	0.047	89.1%	191	0.000	50.0%		
	84	0.041	82.7%	193	0.009	96.8%		
66+95	63	0.000	37.8%	194	0.014	98.7%		
	91	0.079	85.9%	207	195+208	0.005	88.5%	
	67	0.001	62.2%	196+203	0.015	99.4%		
77+110+154	70	0.032	88.5%	197	0.000	50.0%		
	74	0.068	99.4%	199	0.013	98.7%		
	124+135+144	0.067	86.5%	200	0.000	29.5%		
	82+151	0.012	83.3%	205	0.000	30.1%		
	83+119	0.010	90.4%	206	0.007	83.3%		
136+85	87+115+117	0.008	62.8%	Total PCB		1.503	100.0%	

Analytical Methods

All placental samples were maintained in an ultra-cold freezer at -80°C and were prepared for extraction in a Laminar Flow Hood (Nuair - BioGuard). The placental tissues for this study were extracted in a pre-cleaned and combusted (400°C for 1 hour) French Square bottle with Teflon-lined cap. A four surrogate standard (IUPAC 14 (di-CB), 65 (tetra-CB), 166 (hexa-CB), and 209 (deca-CB)) was utilized for each sample as an indicator of method performance and homolog-specific extraction efficiency. A ten-gram placental tissue sample was spiked with the surrogate standard, covered in anhydrous methanol (15-20 mL), and allowed to stand for a period of 20 minutes in a capped French Square bottle. Pre-cleaned anhydrous sodium sulfate (10 grams) was added, and the sample extracted three times each with 15-20 mL hexane using a Brinkman Polytron homogenizer (Model PT 10/35) with small generator (PTA-10S). At the end of the first hexane extraction, 5 mL of deionized water (DI) water was added to the sample to enhance and define the separation of the methanol and hexane phases. Lipid determination was determined from an aliquot of volumetric sample extract by gravimetric procedures. Each of the three fully settled hexane extracts were transferred and combined into a Kuderna-Danish (K-D) apparatus and reduced to 2 mL using a 3-ball Snyder Column on a steam bath. Sample clean-up utilized 10 grams of 4% deactivated Florisil (60/100 PR grade) in a 10x350 mm Chromaflex column, with an upper layer (~ 2 g) of anhydrous sodium sulfate held in place with silanized glass wool. The 2 mL sample extract was added to the Florisil column, eluted with 60 mL of hexane, and concentrated in a K-D apparatus, and brought to a final volume of 1 mL in a Class

A volumetric flask. An internal standard (IUPAC 30) was added prior to gas chromatographic analysis.

Dual-column confirmation of congener-specific PCB, hexachlorobenzene (HCB), p-p' dichlorodiphenyldichloroethylene (DDE), and Mirex analyses were conducted based on capillary column procedures previously described (Pagano et al. 1995; Pagano et al. 1998; Pagano et al. 1999). Briefly, analytical instruments were calibrated every five samples, with a system blank, instrument blank, and calibration check solutions analyzed during each analytical run. A Hewlett-Packard (HP) Model 5890II GC with an electron capture detector (ECD - Ni⁶³) and autosampler was used for data acquisition. The capillary column utilized was a HP Ultra II, 25 meter with 0.22 mm id and 0.33 μ m film thickness. The calibration standard is a 1:1:1:1 mixture of Aroclors 1221, 1016, 1254, and 1260 each at 200 pg/uL, hexachlorobenzene (HCB) at 5 pg/uL, and p-p' DDE and Mirex each at 10 pg/uL (Custom Mix - AccuStandard, Inc.), which allows for the analysis of up to 99 chromatographic zones of 132 congeners/co-eluters (peaks). Dual column confirmational analyses were conducted for each sample with a HP Model 5890 II gas chromatograph with an electron capture detector (Ni⁶³) and autosampler using a 60 meter DB-XLB capillary column with 0.25 mm id and 0.25 μ m film thickness. The calibration standard was a 1:1:1:1:1 mixture of congener mixtures C-CSQ-SETS 1-5 at 10pg/uL per individual congener (C-CSQ-SET; AccuStandard, Inc., New Haven, CT) based on the work of George Frame and co-workers (1996). This analytical setup allows for the analysis of up to 122 chromatographic zones of 155 congeners/co-eluters (peaks). With dual column confirmation, seventy-five (75) peaks were fully confirmed across both analytic columns. All congener values were reported by the laboratory and

were not censored below detection limits (nor imputed as $\frac{1}{2}$ an mdl). This is consistent with our previous work (Stewart et al. 1999, 2000b, 2001), and others (Fitzgerald et al. 2004; Gray et al. 2005). Only those peaks in which the congener was confirmed on both columns were quantified.

Quality Assurance

Laboratory Quality Assurance/Quality Control at the SUNY Oswego ERC is based on a program developed from USEPA protocols (USEPA, 1997). The program consists of replicate analyses, surrogate analyte recoveries (IUPAC 14, 30 IS, 65, 166, and 209), matrix spikes/matrix spike duplicates and, method, reagent and system/instrument blanks at prescribed intervals.

Congener determination, assignments, and accuracy of quantitation were verified for both analytical systems utilizing nine PCB mixtures encompassing all 209 PCB congeners (C-CSQ-SET; AccuStandard, Inc., New Haven, CT) developed by George Frame and co-workers (1996). Calibration tables for each analytical column were constructed based on individual congener amounts, and identified by retention time (RT) match against the C-CSQ-SET standards from AccuStandard. Individual response factors were calculated by dividing these amounts by their corresponding peak areas utilizing external standard calibration algorithms supplied with the HP ChemStation software. In order to assure a stable analytical instrument required for the external calibration method used at the ERC, each analytical run was started with a series of injections of four calibration solutions (N=5, Total=20) of decreasing concentration (6400, 3200, 1600, and 800 pg/uL). The instrument start-up sequence is followed by an initial

calibration, a calibration check, and a final instrument calibration before the start of sample analyses. Analytical calibration standards were run every five samples to update congener retention times and response factors. Instrument calibration was validated at least once during each analytical run with a mid-range standard (400 pg/uL) made by dilution of the stock calibration standard. In addition, at least one system blank (hexane) and instrument blank (no injection) was analyzed during each analytical run to assess system performance. Instrument detection limits (IDL; congener-specific) were established by seven replicate analyses of serial dilutions from the quantitation standards utilized for the analytical system used in this study. Method detection limits (MDL) were based on the assessment of the extent and congener-specific distribution of background contamination using method (procedural) blanks analyzed every five samples. Method blanks encompass all sample preparation and analytical manipulations within an analyte-free matrix.

The procedures above were an update to our previous analytic methodology (Pagano 1995; Stewart et al. 1999) using 89 placental samples. Despite different tissue plugs and a corresponding updated methodology, the correlation for Total PCB between the old and newer values was $r=+.96$, $p<.001$. All samples in the current report were analyzed using the updated method described in this report. There were, however, 8 samples with insufficient tissue to be reanalyzed using the newer standards. Given that the correlations between the original and updated values were quite high ($r=+.96$ for Total PCB; $r = .69$, $p<.001$, $.78$, $p<.001$, and $.98$, $p<.001$ for major PCB congeners 138, 153 and 180, respectively), the values for the remaining 8 subjects were converted to the new metric using a regression slope and intercept with considerable accuracy.

Importantly, all significant regression results reported in the current paper remained significant whether these 8 subjects were included or excluded from the analysis.